Effect of a Lignin-Degrading Fungus on Feeding Preferences of Formosan Subterranean Termite (Isoptera: Rhinotermitidae) for Different Commercial Lumber

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ABSTRACT The feeding preferences of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, for commercial lumber Alaska yellow cedar, *Chamaecyparis nootkatensis* (D. Don) Spach; yellow birch, *Betula alleghaniensis* Britton; northern red oak, *Quercus rubra* L.; redwood, *Sequoia sempervirens* (D. Don) Endl; and spruce (*Picea* spp.) were examined to determine whether the presence of the lignin-degrading basidiomycete *Marasmiellus troyanus* (Murrill) Singer could alter the relative preference of termites for these wood species. In paired choice tests with fungus-inoculated sawdust versus control sawdust, termites showed a strong preference for the fungus-inoculated sawdust for all wood species tested, except for Alaska yellow cedar. In a multiple-choice test using sawdust without fungus, termites showed a very strong preference for red oak sawdust over the other three species. In a paired choice test using fungus-inoculated sawdust, termites showed a preference for redwood over red oak sawdust. In a feeding test using autoclaved wood blocks without fungal decay, there was no difference in termite consumption of birch, red oak, or redwood. The relative preference of termites for redwood increased when blocks were decayed by *M. troyanus* for 3 and 8 wk. These results indicate that chemical modifications due to fungal decay affected the feeding preference of termites for different commercial lumber.

KEY WORDS *Coptotermes formosanus*, wood decay fungi, tunneling behavior, feeding behavior

MANY STUDIES HAVE EXAMINED the feeding preferences of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, for different species of wood (Smythe and Carter 1970, Bultman et al. 1979, Su and Tamashiro 1986, Waller et al. 1990, Morales-Ramos and Rojas 2001). Certain species of wood, such as Alaska yellow cedar, redwood, bald cypress, and teak, are resistant to *C. formosanus* due to the presence of allelochemicals, such as terpenoids, quinones, and phenolics (Waller and La Fage 1987, Scheffrahn et al. 1988, Grace and Yamamoto 1994).

Lignin-degrading fungi alter the chemical composition of the wood (Blanchette 1991). Because lignin-degrading fungi could potentially break down allelochemicals, these fungi could affect the feeding preferences of termites for different wood species. For example, wood consumption and survival of *C. formosanus* was significantly greater on bald cypress decayed by the basidiomycete fungus *Rigidoporus* sp., compared with sound bald cypress, suggesting that chemical modifications in decayed wood improved the suitability of bald cypress as a food source to termites (Waller and La Fage 1987).

The objective of this study was to determine whether the lignin-degrading basidiomycete *Marasmiellus troyanus* (Murrill) Singer influenced the feeding preferences of *C. formosanus* for different commercial lumber species. Formosan subterranean termites have shown a preference for spruce (*Picea* spp.) sawdust or wood blocks inoculated with *M. troyanus* over sawdust or wood blocks without fungus (Cornelius et al. 2002a,b, 2003). In this study, types of commercial lumber were selected that have previously been identified as either preferred or resistant to *C. formosanus.* In this way, we were able to examine whether the presence of *M. troyanus* could alter the relative preference of termites for these wood species.

Termite feeding preferences were examined for the following commercial lumber: spruce, *Picea* spp; yellow birch, *Betula alleghaniensis* Britton; northern red oak, *Quercus rubra* L.; redwood, *Sequoia sempervirens* (D. Don) Endl; and Alaska yellow cedar, *Chamaecyparis nootkatensis* (D. Don) Spach. Spruce was included in this study because all of our previous research examining the effects of *M. troyanus*-inoculated wood on termite behavior was conducted using spruce. Yellow birch and northern red oak were selected because these species were identified as preferred woods in a study where *C. formosanus* was presented with blocks of commercial lumber from 24 wood species in a multiple-choice test. Yellow birch

was the most preferred wood and northern red oak was preferred over 18 other wood species (Morales-Ramos and Rojas 2001). Alaska yellow cedar and redwood were selected because other studies have demonstrated that these wood species have natural resistance to feeding by *C. formosanus* (Su and Tamashiro 1986, Grace and Yamamoto 1994).

Experiments were conducted to examine how *M. troyanus* affected termite preferences for fungus-inoculated sawdust versus sawdust without fungus for each wood species. We also examined termite preferences for sawdust or wood blocks of different wood species with and without the fungus in multiple-choice tests. Multiple choice tests comparing termite feeding on wood blocks of different wood species were conducted when blocks were decayed by *M. troyanus* for either a 3- or 8-wk period before testing. Differences in fungal growth on wood blocks of the different species were examined by determining the weight loss of blocks after 3 or 8 wk of fungal decay.

To determine whether there were differences in the ability of the fungus to colonize sawdust from the different wood species, we estimated the amount of living fungal biomass of *M. troyanus* in the sawdust of each wood species by measuring the ergosterol content of the fungus-inoculated sawdust. Measurements of ergosterol content have been widely used to determine the amount of living fungal biomass in the soil (Davis and Lamar 1992, Eash et al. 1996, Stahl and Parkin 1996). Because ergosterol has been identified as a feeding stimulant (Henderson et al. 1999, Rojas and Morales-Ramos 2001, Cornelius 2003), experiments also were conducted to evaluate the responses of termites to different concentrations of pure ergosterol that was added to sawdust.

Materials and Methods

Collection and Maintenance of Termite Colonies and Fungal Cultures. Formosan subterranean termites were collected from field colonies (C1, C5, C6, C11, and C12) in New Orleans, LA, by using underground bucket traps (Su and Scheffrahn 1986) baited with blocks of spruce wood. Termites were kept in the laboratory in 5.6-liter covered plastic boxes containing moist sand and blocks of spruce wood until they were used in experiments. The litter rot fungus *M. troyanus* (TF-1867) was isolated from leaf litter in an abandoned oil refinery in Darrow, LA (Wunch et al. 1999).

Commercial Lumber. The following types of commercial lumber were obtained from Riverside Lumber Company in New Orleans, LA: spruce, yellow birch, northern red oak, redwood, and Alaska yellow cedar. For each type of lumber used in these tests, sawdust and wood blocks were obtained from the same boards. Commercial lumber of spruce, yellow birch, red oak, and Alaska yellow cedar contained a mixture of heartwood and sapwood. Because the sapwood of redwood is not used in commercial lumber, redwood lumber contained only heartwood.

Inoculation of Sawdust with *M. troyanus*. Potato dextrose agar (PDA) plates were inoculated with *M. troyanus* and placed in incubators set at 25°C with a photoperiod of 12:12 (L:D) h for 4 d. After 4–7 d, 15 (1-cm^2) plugs were removed from the PDA plate and used to inoculate one liter of Sabouraud dextrose broth. The broth was inoculated at ambient temperatures in an orbital shaker at 120 rpm for 7 d. After 7 d, the fungal biomass was strained through a wire mesh strainer, and finally vacuum filtered through a Buchner funnel by using a sterile Whatman no. 4 filter paper. The sawdust was placed in an autoclavable polypropylene vent bag (36 by 61 cm) (Unicorn Imp. & Mfg. Corp., Commerce, TX) with a single 0.2-μm filter (7.6 by 25.4 cm). The bag was heat-sealed and autoclaved vent side up for 60 min on each of two consecutive days. An amount of mycelium equal to 43.4 g of mycelia per 100 g of sawdust was weighed and suspended in 180 ml of sterile water and blended in a Stomacher 400 Mark II Lab Blender (Spiral BioTech, Bethesda, MD) on high for 60 s. After the autoclaved bag was cooled to room temperature, one corner of the bag was cut open, and the filtered mycelium was added to the bag by using a pipette. After the sawdust was inoculated with the filtered mycelium, the opening in the vent bag was heat-sealed. The vent bags were placed in an incubator set at a temperature of 25°C with a photoperiod of 12:12 (L:D) h for 7 d, except that an experiment with Alaska yellow cedar also was conducted after the bags had been kept in the incubator for 3 wk. Bags of sawdust without fungus were prepared using the same procedure (100 g of sawdust in 180 ml of water) to serve as controls.

Extraction of Ergosterol for Quantification of Living Fungal Biomass in Sawdust. To estimate the amount of living fungal biomass in the sawdust of the different wood species, measurements of the ergosterol content in fungus-inoculated bags of sawdust from Alaska yellow cedar, birch, red oak, and redwood were taken after 7 d of fungal growth. In addition, measurements of the ergosterol content in fungus-inoculated bags of Alaska yellow cedar sawdust were taken after 14 and 21 d of fungal growth.

For each wood species, three bags (21 by 21 cm with a 0.2-μm filter [4.5 by 7 cm]) of sawdust (10 g of sawdust per bag) were inoculated with fungus by using the same ratio of mycelium to sawdust as described previously. A 5-g sample of fungus-inoculated sawdust was obtained from each bag after 7, 14, or 21 d of incubation, depending on the experiment. Each sample was rinsed with 30 ml of methanol and 5 ml of KOH/95% ethanol (40 mg/ml). The sample was ultrasonicated in a 60 sonic dismembrator (Fisher Scientific Co., Pittsburgh, PA) for 1 min (power level 3), and then heated in 38 ml of Ace pressure tubes (Sigma-Aldrich, St. Louis, MO) at 85°C for 30 min (mixed at 15 min). After cooling, 10 ml of water was added to a Büchner funnel, and the sample was vacuum filtered through the funnel by using Whatman no. 4 filter paper, with three methanol rinses (10, 10, and 5 ml, respectively). The filtrate was extracted with three 20-ml hexane rinses. The hexane was removed in vacuo and the residue was dissolved in 1.5 ml of methanol and vacuum filtered through Millex-HV

(0.45 μm). Ergosterol from two 1-g samples of fungal biomass was extracted in a similar manner.

Samples and standards were analyzed by high-performance liquid chromatography (HPLC)-UV-mass spectrometry. Injections of 20 μl were used for all samples. Instrumentation consisted of an Alliance HPLC and Micromass ZMD mass spectrometer (Waters, Milford, MA). Chromatography was performed on a Symmetry C18, 3.5 μm, 2.1 by 50-mm column (Waters) with guard column, heated to 35°C. Elution was carried out with a 15-min methanol/water gradient from 70 to 100% methanol at a flow rate of 0.3 ml/min. UV was acquired from 205 to 320 nm. Mass spectrometry parameters were as follows: APCI source; positive ionization mode; corona voltage, 3.5 kV; cone voltage, 30 V; source block temperature, 140°C; APCI probe temperature, 500°C; desolvation gas, 450 liters/min; cone gas, 50 liters/min; and scan range, 250–500 *m/z*.

An ergosterol stock solution was prepared by dissolving 1.30 mg of ergosterol (Sigma-Aldrich) in 60 μl of dichloromethane and diluting to 1.30 ml with methanol (1 mg/ml). Three dilutions were prepared by diluting aliquots of the stock solution with methanol to give 333, 200, and 67 ng/μl. A standard curve of ergosterol was prepared using injections of 1.3, 4.0, and 6.7 μg. The UV (282.2 nm) and MS (379.5 *m/z*) peak areas were determined, and a first order regression curve was applied to the data. Ergosterol amounts in 1-g samples of the fungal mats and 5-g samples of sawdust were determined based on this curve. In a control sample, 375 g of ergosterol was added to 5 g of sawdust. There was 91% recovery of ergosterol in this sample.

Inoculation of Wood Blocks with *M. troyanus*. Wood of each species was cut into small rectangular blocks that were 7 cm in length, 1 cm in width, and 0.5 cm in height. Wood blocks were oven-dried at 90°C for 24 h and then weighed. Each block was soaked in water for 3 d, removed from the water, wrapped in two layers of aluminum foil with a moist paper towel, and autoclaved for 60 min on two consecutive days. After cooling, two blocks of the same wood species were placed side by side in a petri dish (100 by 25 mm) containing PDA. The wood blocks were inoculated with *M. troyanus* by using four (1-cm^2) plugs taken from PDA plates covered with *M. troyanus* mycelium. The plugs were placed on the PDA plates so that they were in contact with both the wood blocks and the PDA. These dishes were placed in an unlit incubator set at a temperature of 25°C for either 3 or 8 wk, depending on the experiment. Wood blocks were removed from the incubator, cleaned, oven-dried at 90°C for 24 h, and weighed to determine the weight loss caused by fungal decay. Weight loss due to fungal decay of blocks of each wood species after 3 wk fungal decay was compared in an experiment by using 14 replicates of each wood species. Weight loss due to fungal decay of blocks of each wood species after 8 wk of fungal decay was compared in an experiment by using 24 replicates of each wood species.

Tunneling Behavior of Termites in Sawdust. Bioassays were conducted using Rubbermaid storage containers (14.5 by 8.5 by 4 cm) (Consolidated Plastics, Twinsburg, OH). Each container contained 100 g of sand (Standard Sand and Silica Company, Davenport, FL) moistened with 20 ml of water. Each container had a 2-cm-diameter hole on each side. A 14-ml (17 by 100-mm) polystyrene round-bottom Falcon test tube (BD Biosciences, Franklin Lakes, NJ) was inserted into each hole and sealed in place using a glue gun. The position of treatment tubes was alternated between replicates to preclude any positional effects. Two hundred termites (190 workers and 10 soldiers) were placed in the center of each container. The termites were able to move freely between the container and the tubes.

For all of the experiments with sawdust, containers were removed from the incubator at the end of the experiment and a rubber stopper was immediately placed in the opening of each tube. Tubes were removed from the container, and the number of termites in each tube was counted. The number of termites in tubes filled with sawdust from each treatment was compared.

Paired Choice Tests with Fungus-Inoculated Sawdust Versus Sawdust without Fungus. For tests with sawdust, each tube was filled with sawdust up to the 10-ml mark on the tube, and 5 ml of distilled water was added to the sawdust in each tube to provide moisture. For paired choice tests of fungus-inoculated sawdust versus control sawdust of each wood species, there were two treatment tubes and two control tubes connected to each container. Treatment tubes were filled with fungus-inoculated sawdust and control tubes were filled with sawdust without fungus. Paired choice tests also were conducted using sawdust from two wood species where the sawdust from one species was fungus-inoculated and the sawdust of the other species was not inoculated with fungus. For the paired choice tests, there were eight replicates, with two replicates each from four termite colonies. These experiments lasted for 18–22 h.

Paired Choice Tests with Sawdust Treated with Ergosterol versus Untreated Sawdust. For paired choice tests comparing termite preferences for sawdust treated with an extract of pure ergosterol (Sigma-Aldrich) versus untreated sawdust, red oak sawdust (50 g) was placed in a 1000-ml round-bottom flask, and 100 ml of a methylene chloride solution of ergosterol (0.025, 0.05, 0.2, or 0.4 mg/ml) was added. The solution was stirred, and the solvent was removed in vacuo while the flask was slowly rotated. Tests were conducted with concentrations of 50, 100, 400, or 800 μg of ergosterol per gram of sawdust. Control sawdust was prepared with methylene chloride only. Each tube was filled with an 8-ml volume of red oak sawdust. There were eight replicates, with two replicates each from four termite colonies. These experiments lasted for 18–22 h.

Multiple-Choice Tests with Sawdust from Four Wood Species. For multiple-choice tests with sawdust from four wood species, each tube was filled with sawdust

of a different species up to the 10-ml mark on the tube. The position of the different species was alternated between replicates to preclude any positional effects. These tests were conducted using both fungus-inoculated sawdust and sawdust without fungus. A choice test also was conducted comparing fungus-inoculated red oak versus fungus-inoculated redwood where two tubes in each replicate were filled with red oak sawdust and two tubes were filled with redwood sawdust. There were 12 replicates, with three replicates each from four termite colonies. For these multiple-choice tests, the experiments lasted for 3 d, and the distance that termites tunneled into each tube was measured daily.

Wood Consumption of Blocks from Four Wood Species. These bioassays were conducted using the same testing apparatus as the bioassays with sawdust. Two hundred termites (190 workers and 10 soldiers) were placed in the center of each container. The termites were able to move freely between the container and the tubes. Wood blocks (7 by 1 by 0.5 cm) were oven-dried at 90°C for 24 h and weighed. For experiments using fungus-inoculated blocks, decayed wood blocks were cleaned, oven-dried, and weighed to determine weight loss due to fungal decay before using blocks in feeding bioassays.

A wood block was placed in each tube. Each of the four tubes contained a block from a different wood species. There were 12 replicates, with three replicates each from four termite colonies. These experiments lasted for 3 wk and at the end of each experiment, the number of termites in each replicate was counted. Wood blocks were cleaned and oven-dried at 90°C for 24 h and then weighed. Weight loss due to termite feeding on decayed blocks was determined by comparing wood weights taken after blocks were decayed for either 3 or 8 wk with weights of wood blocks at the end of the feeding bioassays.

Multiple-choice tests were conducted using wood blocks of birch, red oak, redwood, and Alaska yellow cedar where blocks underwent the following treatments before testing: 1) blocks were oven-dried only; 2) blocks were autoclaved for 60 min on two consecutive days by using the same procedure used for the fungus-inoculated blocks; 3) blocks were autoclaved for 60 min on two consecutive days, inoculated with fungus as described previously, and decayed for 3 wk; and 4) blocks were autoclaved for 60 min on two consecutive days, inoculated with fungus as described previously, and decayed for 8 wk. For this test, there were six replicates each from four termite colonies.

Statistical Analysis. In tests using sawdust, the number of termites in each tube was counted. In paired choice tests, numbers of termites in tubes were compared, in a pooled data set of the four colonies, by using a *t*-test for matched pairs. In tests using wood blocks, the weight loss of blocks due to both fungal decay and termite feeding was compared for each wood species. Weight loss of blocks of different wood species due to fungal decay was compared using a one-way analysis of variance (ANOVA). Differences were compared using a Tukey's honestly significant difference (HSD) test (SPSS Inc. 1996).

In the four-way multiple choice tests with both sawdust and wood blocks, data were analyzed using a two-way ANOVA where wood species and colony were factors. In cases where there was no significant interaction between wood species and colony and either factor was significant, differences were compared using a Tukey's HSD test (SPSS Inc. 1996).

In the four-way choice tests using wood blocks, termite survival after 3 wk was determined. Proportional survival data were transformed by the arcsine of the square root. A one-way ANOVA on survival data were conducted to determine if there were significant colony differences in termite survival.

Table 1. Mean (± SEM) number of *C. formosanus* in tubes filled with fungus-inoculated sawdust versus sawdust without fungus in a paired choice test after 18–22-h exposure

Wood species[a]	No. of termites in tubes	
	Fungus-inoculated	No fungus
Spruce	137.4 ± 7.9	6.0 ± 2.1**
Birch	140.3 ± 10.2	3.4 ± 1.3**
Red oak	113.0 ± 7.4	24.0 ± 4.3**
Redwood	150.1 ± 8.5	11.8 ± 4.1**
Alaska yellow cedar	7.6 ± 3.8	16.9 ± 10.5
Alaska yellow cedar (3 wk)	14.5 ± 11.1	5.4 ± 3.6

** $P < 0.01$.

[a] Bags of fungus-inoculated sawdust and control bags were kept in a incubator for 7 d before testing, except for one experiment with Alaska yellow cedar where bags of sawdust were kept in an incubator for 3 wk before testing.

Results

Tunneling Behavior of Termites in Sawdust. *Paired Choice Tests with Fungus-Inoculated Sawdust versus Sawdust without Fungus.* In paired choice tests with fungus-inoculated sawdust versus control sawdust, termites showed a strong preference for the fungus-inoculated sawdust for all wood species tested, except for Alaska yellow cedar. There were low numbers of termites in tubes in both the test where fungus-inoculated sawdust of Alaska yellow cedar was kept in the incubator for 7 d, and the test where the fungus-inoculated sawdust was kept in the incubator for 3 wk (Table 1). In the paired choice tests where sawdust from one wood species was inoculated with fungus and sawdust from the other species was not inoculated with fungus, termites preferred fungus-inoculated birch over red oak without fungus, but termites preferred red oak without fungus over fungus-inoculated Alaska yellow cedar (Table 2).

Paired Choice Tests with Sawdust Treated with Ergosterol versus Untreated Sawdust. When the amount of ergosterol was measured from the fungal mycelium by itself, the ergosterol content was measured at 300 μg of ergosterol per gram of fungal mycelium. The measurements of the ergosterol content of sawdust from four wood species determined that there were significant differences between wood species in the amount of fungal biomass after 7 d of fungal growth

Table 2. Mean (± SEM) number of *C. formosanus* in tubes filled with fungus-inoculated sawdust versus sawdust without fungus in a paired choice test after 18–22-h exposure

Wood species	No. of termites in tubes	
	Fungus-inoculated	No fungus
Birch/red oak	95.8 ± 18.0	12.5 ± 4.1**
Alaska yellow cedar/red oak	16.0 ± 7.4	64.8 ± 15.4*

* $P \leq 0.05$; ** $P \leq 0.01$.

Table 4. Mean (± SEM) number of *C. formosanus* in tubes filled with sawdust treated with an extract of ergosterol or the solvent control in a paired choice test after 18–22-h exposure

Ergosterol conc. (μg/g sawdust)	No. of termites in tubes	
	Treated	Control
50	36.4 ± 8.5	43.1 ± 12.8
100	19.2 ± 6.8	38.7 ± 12.2
400	36.6 ± 9.5	20.1 ± 5.9
800	51.6 ± 8.4	43.6 ± 7.1

($F = 7.20$; df = 3, 8; $P = 0.012$). The ergosterol content of red oak sawdust was significantly greater than the ergosterol content in birch and Alaska yellow cedar (Table 3). The measurements of ergosterol content of Alaska yellow cedar were not significantly different after 14 and 21 d of fungal growth ($F = 0.62$; df = 1, 4; $P = 0.50$) (Table 3). Ergosterol content of Alaska yellow cedar sawdust was lower in the experiment where incubation lasted for 14 or 21 d than in the experiment where incubation lasted for only 7 d, indicating that the fungus was not able to survive on Alaska yellow cedar sawdust.

In paired choice tests where ergosterol was added to red oak sawdust versus solvent-treated sawdust, termites showed no preference for sawdust with ergosterol at any of the concentrations tested (Table 4).

Multiple-Choice Tests with Sawdust from Four Wood Species. In the multiple-choice tests using sawdust from birch, spruce, red oak, and redwood, there were no significant effects of the interaction of wood species and colony in the test with control sawdust, but there was a significant interaction in the test with fungus-inoculated sawdust (Table 5, experiment 1). There were no significant colony differences in any of the tests using sawdust (Table 5).

In the test with the control sawdust, termites tunneled all the way through the red oak in virtually all of the replicates (6.5 cm) within 3 d, but they tunneled an average of only 2 cm or less through the sawdust from the other wood species (Fig. 1A). In the test with fungus-inoculated sawdust, termites tunneled through virtually all of the tubes filled with fungus-inoculated sawdust, regardless of species, within 3 d (Fig. 1B). In the test with the control sawdust, termites showed a very strong preference for red oak sawdust after 3 d (Fig. 2A). In the test with the fungus-inoculated sawdust, differences in the number of termites in tubes with sawdust of the four wood species could not be analyzed separately because of the significant interaction effect of wood species and colony (Table 5, experiment 1). A comparison of the average numbers of termites in tubes showed that there were more termites from C1 in the red oak sawdust, and there were more termites from the other three colonies, especially C5, in the redwood sawdust (Fig. 2B).

Table 3. Mean (± SEM) amount of ergosterol (micrograms per gram of sawdust) from sawdust of four wood species

Wood species	No. of days after inoculation	Ergosterol content (μg)
Experiment 1		
Birch	7	61.77 ± 9.7a
Red oak	7	97.30 ± 5.0b
Redwood	7	65.5 ± 4.9ab
Alaska yellow cedar	7	52.8 ± 8.0a
Experiment 2		
Alaska yellow cedar	14	21.43 ± 4.1a
Alaska yellow cedar	21	25.2 ± 2.4a

Means followed by the same letters, within an experiment, are not statistically different, Tukey HSD ($P = 0.05$).

In a test directly comparing the preference of termites from the four colonies for fungus-inoculated red oak sawdust versus fungus-inoculated redwood in a paired choice test, there was no significant interaction between wood species and colony, or any significant differences between colonies, but there was a significant difference between the two types of sawdust (Table 5, experiment 3). There were significantly more termites in tubes filled with redwood compared with red oak (Fig. 3A), and termites from all four colonies showed a significant preference for redwood over red oak (Fig. 3B).

Wood Consumption of Blocks from Four Wood Species. In multiple-choice tests with wood blocks of Alaska yellow cedar, birch, red oak, and redwood, there were no significant effects of the interaction between wood species and colony (Table 5). In the test using blocks without fungal decay that were not autoclaved, termites showed a significant preference for birch and red oak over Alaska yellow cedar and redwood (Fig. 4A). In the test using blocks without fungal decay that were autoclaved following the same procedure used for fungus-inoculated blocks, there was no significant difference in the wood consumption of birch, red oak, and redwood, but there was a significant preference for birch and redwood over Alaska yellow cedar (Fig. 4B).

When wood blocks were decayed for 3 wk, there was no detectable weight loss due to fungal decay in wood blocks of any of the four wood species. In the feeding bioassay using wood blocks that had been decayed for 3 wk, termites showed a significant preference for redwood over all three of the other wood species (Fig. 4C). There was a significant difference in the consumption rates of the termite colonies (Table 5, experiment 6), where C6 consumed significantly more wood than C12 overall.

When wood blocks were decayed for 8 wk, there were significant differences in the weight loss of blocks of the different wood species due to fungal

Table 5. Analysis of variance for multiple-choice tests with sawdust and blocks of different wood species

Source of variation[a]	*F*	df	*P*
Multiple-choice tests with sawdust: two-way ANOVA			
1. Sawdust without fungus: B, RO, RW, S			
Wood species	64.1	3, 32	<0.0001**
Colony	0.19	3, 32	0.9
Wood species*colony	0.55	9, 32	0.83
2. Fungus-inoculated sawdust: B, RO, RW, S			
Wood species	31.1	3, 32	<0.0001†
Colony	0.15	3, 32	0.92
Wood species*colony	8.1	9, 32	<0.0001**
3. Fungus-inoculated sawdust: RO, RW			
Wood species	17.3	1, 8	0.003**
Colony	1.5	3, 8	0.30
Wood species*colony	0.76	3, 8	0.55
Multiple-choice tests with wood blocks			
4. Multiple-choice test using control wood blocks without fungus: AC, B, RO, RW			
Two-way ANOVA: weight loss from wood blocks after 3-wk feeding test			
Wood species	9.2	3, 32	<0.0001**
Colony	0.72	3, 32	0.55
Wood species*colony	0.23	9, 32	0.99
One-Way ANOVA: survival of termites after 3-wk feeding test			
Colony	1.11	3, 8	0.40
5. Multiple-choice test using autoclaved wood blocks without fungus: AC, B, RO, RW			
Two-way ANOVA: weight loss from wood blocks after 3-wk feeding test			
Wood species	5.5	3, 32	0.004**
Colony	1.4	3, 32	0.25
Wood species*colony	0.60	9, 32	0.81
One-way ANOVA: survival of termites after 3-wk feeding test			
Colony	1.94	3, 8	0.20
6. Multiple-choice test using fungus-inoculated wood blocks (3-wk decay): AC, B, RO, RW[b]			
Two-way ANOVA: weight loss from wood blocks after 3-wk feeding test			
Wood species	12.9	3, 28	0.04*
Colony	3.1	3, 28	<0.001**
Wood species*colony	1.2	9, 28	0.31
One-way ANOVA: survival of termites after 3 wk			
Colony	0.84	3, 7	0.51
7. Multiple-choice test using fungus-inoculated wood blocks (8-wk decay): AC, B, RO, RW			
Two-way ANOVA: weight loss from wood blocks after 3-wk feeding test			
Wood species	24.4	3, 76	0.0001**
Colony	1.8	3, 76	0.152
Wood species*colony	0.91	9, 76	0.52
One-way ANOVA: survival of termites after 3-wk feeding test			
Colony	9.4	3, 19	0.001**

* $P \leq 0.05$; ** $P \leq 0.01$; † Factor could not be analyzed separately due to significant interaction effect.
[a] AC, Alaska yellow cedar; B, birch; RO, red oak; RW, redwood; S, spruce.
[b] Only two replicates from C5 were included in the analysis for this test because termites escaped from one of the C5 replicates.

decay ($F = 32.3$; df = 3, 92; $P < 0.0001$). Weight losses due to fungal decay were greater in red oak blocks than in the other three species, and there was virtually no weight loss in Alaska yellow cedar blocks after 8-wk exposure to the fungus (Table 6).

In the feeding bioassay using wood blocks that had been decayed for 8 wk (Table 5, experiment 7), consumption of birch and redwood was significantly greater than consumption of red oak and Alaska yellow cedar (Fig. 4D). There was a significant difference in the survival of termites from the four colonies (Table 5, experiment 7), where C12 had a significantly greater mortality rate than the other three colonies ($P = 0.001$).

Discussion

Formosan subterranean termites showed an overwhelming preference for fungus-inoculated sawdust over control sawdust in paired choice tests using birch, spruce, red oak, and redwood. However, termites did not tunnel into either fungus-inoculated or control sawdust of Alaska yellow cedar. Termites probably avoided tunneling into sawdust of Alaska yellow cedar because of the presence of allelochemicals, such as nootkatone, which are repellent to termites (Bläske and Hertel 2001, Maistrello et al. 2001, Zhu et al. 2001).

The amount of living fungal biomass, estimated by measuring the ergosterol content of the sawdust, was not significantly different in Alaska yellow cedar than in birch and redwood after 7 d of fungal growth. Therefore, the lack of termite tunneling activity in fungus-inoculated Alaska yellow cedar sawdust cannot be explained entirely by an absence of fungus on this substrate. However, the amount of ergosterol in Alaska yellow cedar did not increase between 14 and 21 d of growth and was lower than the amount found after 7 d growth. The decrease in ergosterol content

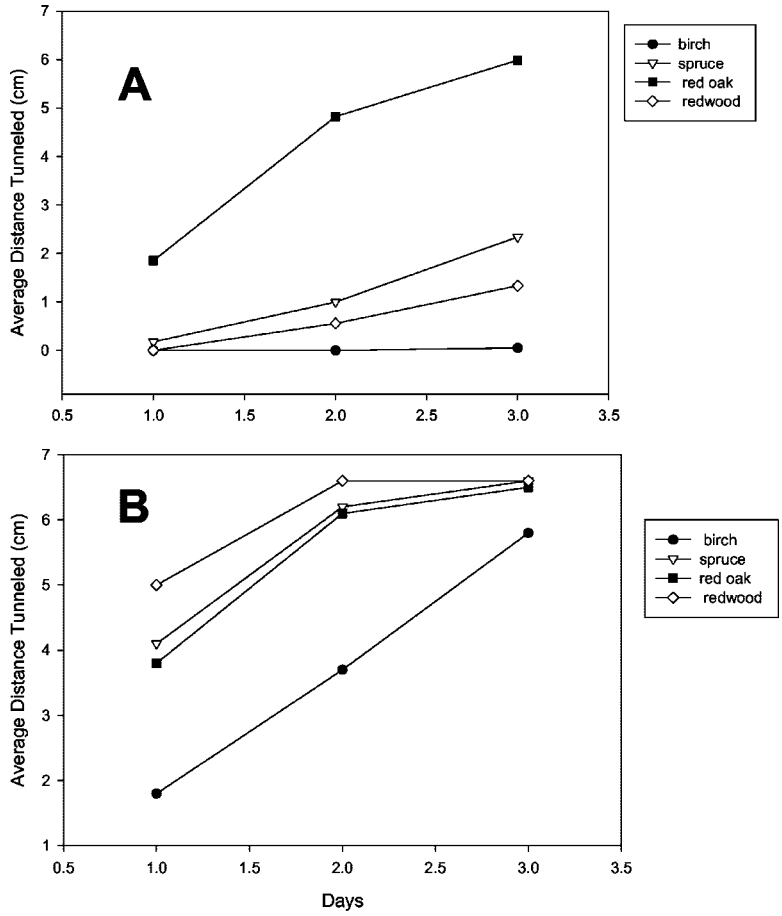

Fig. 1. Average distance (centimeters) tunneled by termites in tubes containing sawdust of four wood species. (A) Without fungus. (B) Inoculated with *M. troyanus*.

indicates that *M. troyanus* could not survive on the Alaska yellow cedar, presumably due to the presence of allelochemicals in the sawdust.

With the exception of Alaska yellow cedar, tunneling activity by termites was much greater in fungus-inoculated sawdust than in control sawdust. Other studies have identified ergosterol as a feeding stimulant for *C. formosanus* (Henderson et al. 1999, Rojas and Morales-Ramos 2001, Cornelius 2003). However, the increased activity of termites in fungus-inoculated sawdust does not seem to be due to the presence of ergosterol in the sawdust. Termite activity was not affected by adding ergosterol to sawdust, even though the concentrations tested were similar to concentrations of ergosterol found in fungus-inoculated sawdust. It is not surprising that the tunneling activity of termites in sawdust was not affected by the presence of ergosterol given that ergosterol, as a component of the cell walls of most types of fungi, is an integral part of the soil matrix. Hence, termites would encounter ergosterol in virtually any soil type where they were constructing a gallery system. In an experiment where termite consumption of ergosterol-treated and solvent-treated filter paper disks was measured, ergosterol acted as a feeding stimulant when a specific concentration was applied to the filter paper (Cornelius 2003). Bioassays which identify compounds as feeding stimulants based on increased consumption of filter paper within a narrow range of concentrations may have limited value in the real world.

In the multiple-choice test comparing birch, spruce, red oak, and redwood sawdust without fungus, termites showed an overwhelming preference for red oak sawdust. This preference for red oak sawdust may have been due primarily to differences in the texture of the sawdust that affected the tunneling behavior of termites. Consumption rates of red oak and birch wood blocks were not different in the multiple-choice test comparing control blocks of four wood species (sawdust was collected from the same wood that was used to cut the blocks). Therefore, the results of termite feeding tests on control wood blocks of these species indicate that the strong preference for tunneling within red oak sawdust may have been related primarily to textural differences that affect the tunneling behavior of termites, rather than to their feeding behavior.

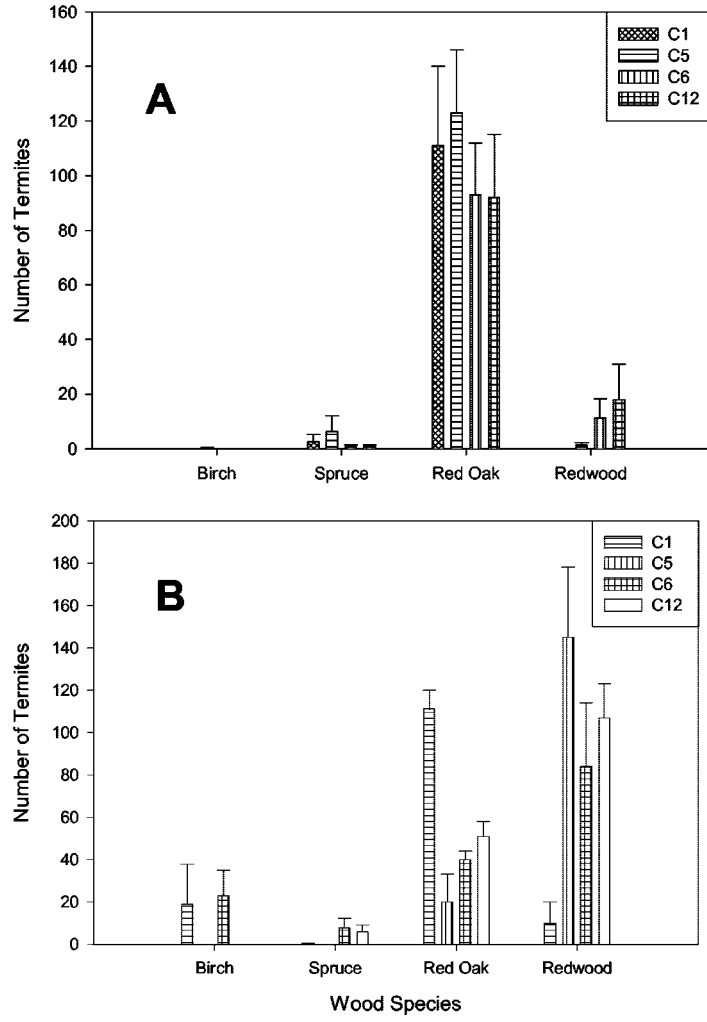

Fig. 2. Mean ± SEM number of termites in tubes containing sawdust of four wood species. (A) Without fungus. (B) Inoculated with *M. troyanus*.

Tunneling activity of termites in the control sawdust of the four wood species differed dramatically. In virtually every replicate, termites immediately began tunneling into red oak sawdust and tunneled all the way through most of the sawdust-filled tubes within 3 d, whereas termites tunneled less than half-way through tubes filled with sawdust of the other three wood species. In the multiple-choice test with fungus-inoculated birch, spruce, red oak and redwood sawdust, termites immediately began tunneling into sawdust of all four species and tunneled all the way through sawdust-filled tubes in most of the replicates within 3 d. In a previous study, termites tunneled through sand treated with a crude extract of fungus-inoculated spruce sawdust more readily than through sand treated with an extract of control sawdust, indicating that termites were responding to chemical changes in the fungus-inoculated sawdust (Cornelius et al. 2002a,b). In this study, the increase in tunneling activity by termites in fungus-inoculated sawdust could be due either to textural changes in the sawdust, chemicals produced by the fungus, or to the metabolism by the fungus of chemicals in the sawdust that act as deterrents to termites.

In the multiple-choice tests using fungus-inoculated sawdust, there was a significant interaction effect between colony and wood species where there were more termites from C1 in the fungus-inoculated red oak, but more termites from the other three colonies in the fungus-inoculated redwood. However, in a paired choice test, termites from all four colonies showed a significant preference for fungus-inoculated redwood sawdust over fungus-inoculated red oak sawdust. In the four-way choice test with fungus-inoculated sawdust, termites explored all four tubes but tended to aggregate in tubes containing either red oak or redwood. Termites tend to aggregate within a particular tube in any given replicate. The large numbers of C1 termites in red oak in the four-way choice test is more likely to be the result of the tendency of

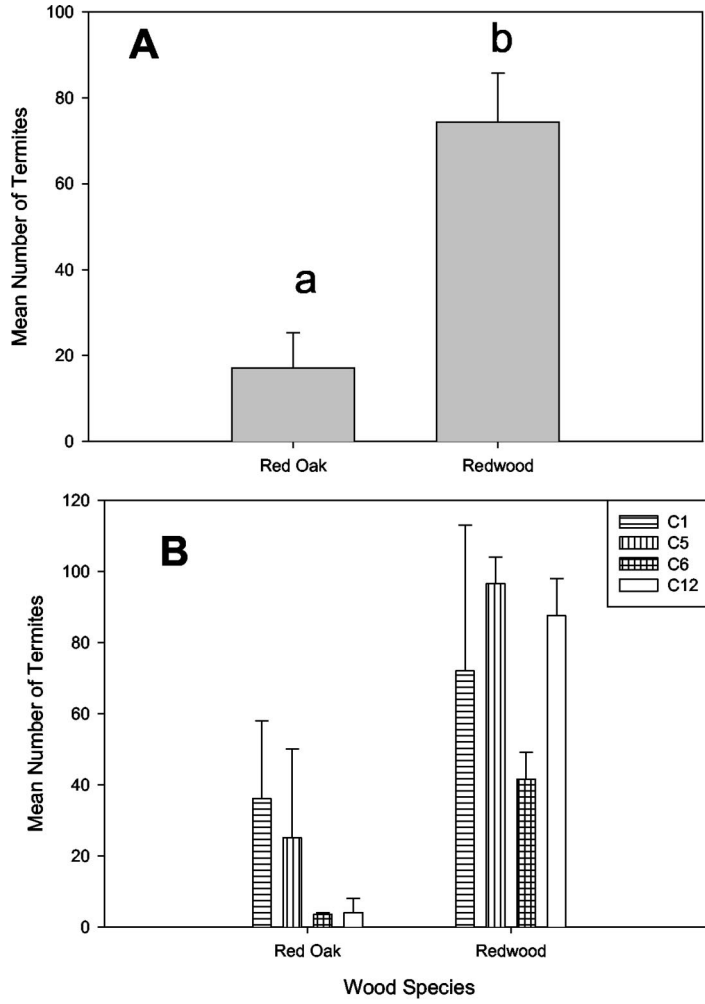

Fig. 3. Mean ± SEM number of termites in tubes containing fungus-inoculated sawdust of red oak or redwood in a paired choice test. (A) Total number of termites. (B) Number of termites in each colony.

termites to aggregate on an acceptable food source than the result of an actual colony difference in termite preferences for sawdust.

There were significant differences in wood consumption of blocks of the four wood species, depending on the treatment. The heartwood of redwood and Alaska yellow cedar has natural resistance to Formosan subterranean termites (Grace and Yamamoto 1994). Because Alaska yellow cedar blocks contained some sapwood that is less resistant, there was some feeding damage on Alaska yellow cedar blocks even in the feeding tests using control blocks. When wood blocks without fungal decay were not autoclaved, termites fed significantly less on Alaska yellow cedar and redwood. When blocks without fungal decay were autoclaved, termites fed significantly less on Alaska yellow cedar but not redwood. The increase in the relative feeding preference for redwood when blocks were autoclaved indicates that the high temperatures caused chemical changes in the wood that affected the feeding behavior of termites.

Even though there was no detectable weight loss due to fungal decay for any of the wood species in the experiment where wood blocks were decayed by *M. troyanus* for 3 wk before testing, relative feeding preferences of termites for redwood were affected. In this feeding test, termites consumed significantly more redwood than any of the other wood species, indicating that fungal hyphae may have already started penetrating into the wood.

When wood blocks were decayed by *M. troyanus* for 8 wk, weight loss due to fungal decay was greater in red oak blocks than in the three other species, and weight loss in redwood and birch was similar. In the feeding test, termites consumed significantly more birch and redwood than red oak and Alaska yellow cedar. After 8 wk of fungal decay, the relative feeding preference for red oak was much less than in any of the other

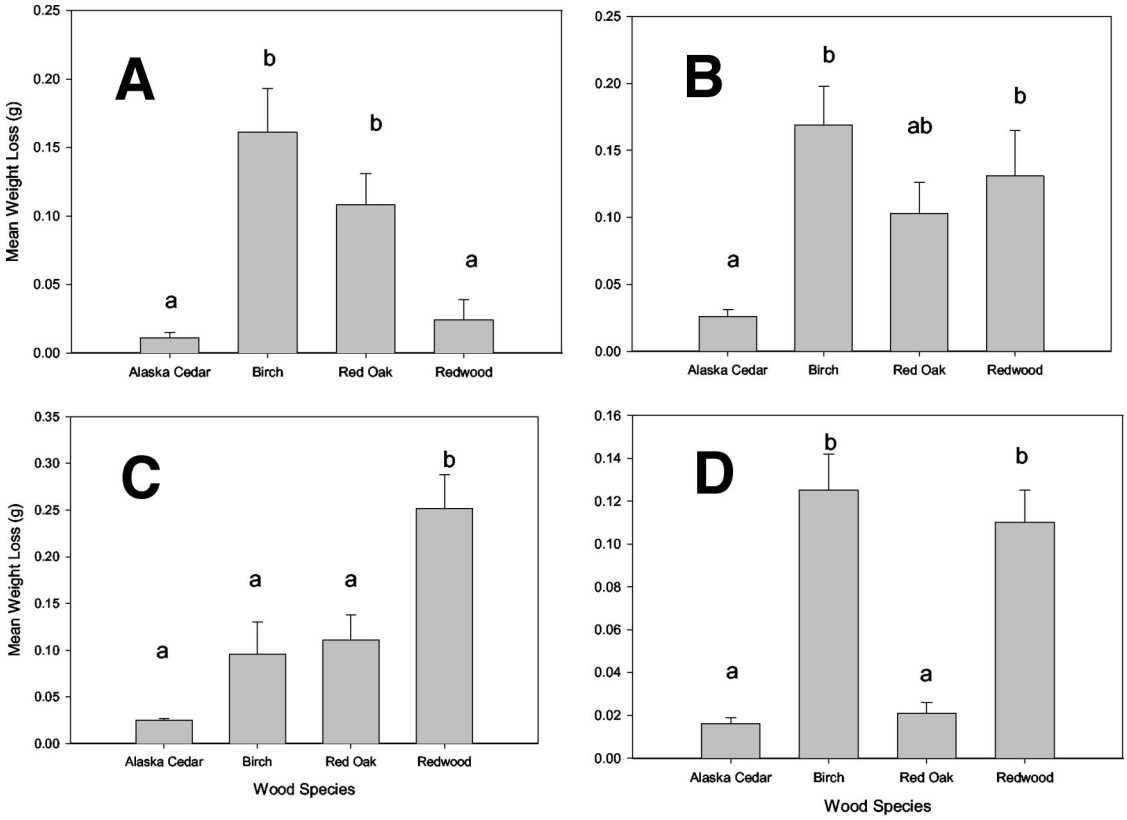

Fig. 4. Mean ± SEM weight loss (grams) of wood blocks from four wood species after 3 wk of feeding by termites. (A) Before testing, wood blocks were oven-dried only. (B) Before testing, wood blocks were oven-dried and autoclaved. (C) Before testing, wood blocks were decayed by *M. troyanus* for 3 wk. (D) Before testing, wood blocks were decayed by *M. troyanus* for 8 wk.

feeding tests. Because weight loss due to decay by *M. troyanus* was significantly greater in red oak than the other three wood species after 8 wk, it is possible that the red oak blocks had become too heavily decayed for the termites. In a study where spruce blocks were decayed by *M. troyanus* over a 12-wk period, termites showed a significant preference for decayed blocks over control blocks after 8 wk, but not 12 wk, when blocks were kept in an incubator under a 24-h dark cycle (Cornelius et al. 2003). These results indicate that termites prefer moderately decayed wood, but not heavily decayed wood, over sound wood.

The relative preference of termites for redwood sawdust and blocks was affected by the presence of the fungus *M. troyanus.* In the presence of the fungus, termites shifted their preference from red oak sawdust to redwood sawdust. Also, relative consumption rates of decayed redwood blocks were greater than consumption rates on undecayed redwood blocks. Chemical modifications due to fungal decay may have affected termite behavior. Further research is necessary to elucidate how these chemical changes due to decay by *M. troyanus* influence termite behavior and whether the fungus is breaking down allelochemicals in redwood that act as deterrents to termites.

Table 6. Mean (± SEM) weight loss (milligrams) due to fungal decay of wood blocks from four wood species exposed to *M. troyanus* for 8 wk

Wood species[a]	Weight loss of wood blocks (mg)
Alaska yellow cedar	5.7 ± 1.3a
Birch	28.8 ± 3.7b
Red oak	52.8 ± 4.11c
Redwood	25.9 ± 3.7b

Means followed by the same letters are not statistically different, Tukey HSD ($P = 0.05$).

[a] There were 24 replicates of each wood species for this experiment.

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